DATA EVALUATION RECORD
MYSID CHRONIC TOXICITY TEST
GUIDELINE OPPTS 850.1350

1. **CHEMICAL:** Bifenthrin PC Code No.: 128825

2. **TEST MATERIAL:** Bifenthrin Purity: 93.6%

3. **CITATION** Marini, J. (2014) Bifenthrin- Life Cycle Toxicity Test with Mysid (Americamysis bahia) Following Draft OCSPP Guideline 850.1350. Project Number: 14011/6116, 021712/OCSPP/MYSID/FLC, 14011/6105. Unpublished study prepared by Smithers Viscient Laboratories.

MRID No.: 49412102
DP Barcode: D421505

4. **REVIEWED BY:** John Marton, Ph.D., Environmental Scientist, CDM Smith

Signature: **Date:** 11/23/14

APPROVED BY: Teri S. Myers, Ph.D., Environmental Scientist, CDM Smith

Signature: **Date:** 06/03/15

5. **APPROVED BY:** Brian Montague, Fisheries Biologist **Date:** August 12, 2015
ERB5/EFED/OPP/OSCPP

6. **STUDY PARAMETERS**

Age of Test Organism:	Neonates, ~24 hours old
Definitive Test Duration:	28 days
Study Method:	Flow-through
Type of Concentrations:	Mean-measured

7. **CONCLUSIONS:**

Results Synopsis
NOAEC: 1.6 ng ai/L
LOAEC: >1.6 ng ai/L

Endpoint(s) Affected: none
Most sensitive endpoint(s): none

8. **ADEQUACY OF THE STUDY**

A. Classification: This study is scientifically sound and is classified as supplemental. Repetition of the study is not required.

B. Rationale: The purpose of this study is to establish effect parameters for growth and reproduction. The concentration levels selected for this study failed to produce useable chronic endpoints for risk assessment purposes. However, the maximum nominal concentration (1.6 ng ai/L) selected for this study was equivalent to the NOAEC observed for survival in acute 96 hour testing (MRID 49060102). In preliminary testing some effects were noted at the 1.5 ng ai/L nominal level and were the basis for the range selection used in the definitive test. A 3.1 ng ai/L nominal concentration produced 15% mortality in the acute study and presumably would have done the same for the F0 adults in a chronic study. Therefore repetition at a higher concentration range might not allow adequate parental survival before reproduction could occur. Preliminary testing results for the study are posted in the appendices of this review for further reference.

C. Repairability: Not repairable

9. MAJOR GUIDELINE DEVIATIONS:

- The photoperiod (16 hr light:8 hr dark) deviated slightly from recommendations (14 hr light:10 hr dark).
- Excessive analytical variation (CV, 25-49%) was observed at all treatment levels.
- The body length of each mysid was not recorded at the time of sexual discernment (Day 13).
- The time to first brood release was not evaluated as a toxicological endpoint.
- Selection of concentration range for the definitive study failed to produce statistically significant chronic effects despite observations of effects observed in the preliminary testing.

10. MATERIALS AND METHODS:

A. Biological System

Guideline Criteria	Reported Information
Species: An estuarine shrimp species, preferably *Americamysis bahia*.	*Americamysis bahia*
Duration of the Test: 28 days	28 days

Guideline Criteria	Reported Information
Source (or supplier) Should originate from in-house cultures.	In-house cultures. The brood stock originated from MBL Aquaculture (Sarasota, FL) and was maintained in-house for *ca.* 26 months prior to use.
Parental Acclimation Within a 24-h period, changes in water temperature should not exceed 1°C, while salinity changes should not exceed 5% Mysids should be in good health.	Mysids were cultured in six re-circulating 80-L glass aquaria in dilute, natural seawater (same as that used during the definitive study). During the 14-day period prior to testing, the seawater was characterized as having a salinity range of 20 to 22‰, pH of 7.7 to 7.9, and DO range of 90 to 96% saturation. The cultures were maintained under a 16-hr light (26 to 59 foot candles)/8-hr dark photoperiod at 26°C. Feeding was not described. The culture organisms did not show any sign of sickness, disease, injuries, or abnormalities, and the brood stock and test organisms were determined to be in good health at test initiation.
Chamber Location: Treatments should be randomly assigned to test chamber locations.	Organisms were impartially selected and distributed to retention chambers, and the retention chambers were placed in their respective exposure aquaria.

Guideline Criteria	Reported Information
Distribution: **No. of mysids before pairing**: Minimum of 40 mysids per concentration **No. of mysids after pairing**: Mysids should be separated into replicate groups of no more than eight individuals when most of the mysids reach sexual maturity (usually 10 to 14 days after test initiation).	80/treatment level: 20 mysids per retention chamber, one chamber per aquarium, and four replicate aquaria per treatment level. ≤20 pairs/level: one pair per pairing chamber, up to five pairs per replicate, and four replicate aquaria per treatment level. Unpaired mysids were pooled and maintained in one of the initial retention chambers until they were paired or until test termination. Male mysids from the pooled excess organisms were used to replace dead males from the paired groups; females that died in the pairing chambers were replaced at the Study Director's discretion. Once reproduction within the study had begun, females were not replaced.
Pairing: Should be conducted when most of the mysids are sexually mature (usu. 10-14 days after test initiation)	Day 13
Offspring Exposure: Live young must be counted and separated into retention chambers at the same concentration where they originated.	40/treatment level: when possible*, groups of 10 offspring per replicate, with four replicate aquaria per treatment level were placed in their respective exposure aquaria and maintained for 96 hours.
Observations:	- Adult mysids were observed daily for mortality, sub-lethal effects, and reproduction (post-pairing). - Offspring were observed daily for mortality and sub-lethal effects.

Guideline Criteria	Reported Information
Feeding: Mysids should be fed during testing. A recommended food is live *Artemia* spp. Nauplii (*ca.* 48-hr old).	Mysids were fed live brine shrimp (*Artemia salina*) nauplii (≤48-hours old) twice daily. At least one of these feedings was with brine shrimp nauplii enriched with Selco® (a supplemental substance high in saturated fatty acids). F_0-generation Days 0-3: 90 nauplii/mysid Days 4-6: 135 nauplii/mysid Days 7-9: 180 nauplii/mysid Days 10-13: 225 nauplii/mysid Days 13+ (pairing chambers): *ca.* 450 nauplii/mysid Days 13+ (retention chambers): *ca.* 3600 nauplii/chamber F_1-generation *ca.* 90 nauplii/mysid
Controls: Negative control and carrier control (when applicable) are required.	Negative control group was included.

Comments: The maximum organism loading concentration (based on a typical average wet weight of 4.5 mg per adult mysid) did not exceed 0.0025 g of biomass/L of flowing test solution per day.

The in-life portion of the definitive toxicity test was conducted from February 6 to March 6, 2014.

B. <u>Physical System</u>:

Guideline Criteria	Reported Information
Test Item(s):	Identity: Bifenthrin Synonym: Bifenthrin Technical IUPAC name: not reported CAS name: not reported CAS no.: 82657-04-3 Description: not reported Lot no.: PL09-0251 Purity: 93.6% Storage: room temperature, refrigerated
Test Water: May be natural or artificial seawater. Natural seawater should be filtered (>20 µm). Artificial seawater should be prepared with deionized (conductivity <0.1 mS/M at 12°C) or glass-distilled water. When deionized water is prepared from a natural water source, conductivity and TOC (or COD) should be measured in each batch.	Dilution water consisted of diluted, filtered natural seawater. Seawater was pumped from the Cape Cod Canal (Bourne, MA) from *ca.* 1-4 meters offshore at a depth of *ca.* 0.5 to 3 meters. In the laboratory, the seawater was adjusted to a salinity of 20 ± 3‰ with laboratory well water and filtered (20- and 5-µm) prior to use. The seawater used for this study had a salinity range of 20 to 22‰ and pH range of 7.6 to 8.0. The TOC of the dilution water was 1.1 and 1.2 mg/L for February 2014 and March 2014, respectively. Results of periodic analysis for pesticides, organics, and metals indicated that none of these compounds were detected at concentrations that are considered toxic in any of the water samples analyzed.
Salinity: 20 ± 3‰ (parts per thousand). Should be measured weekly in each chamber.	19 to 22‰ Measured in all replicates on Day 0, and in alternating replicates from each level daily thereafter.

Guideline Criteria	Reported Information
pH: Should be measured weekly in each chamber.	7.6 to 8.0 Measured in all replicates on Day 0, and in alternating replicates from each level daily thereafter.
Dissolved oxygen: Should remain between 60 and 105% saturation. Should be measured weekly in each chamber.	4.96 to 7.09 mg/L (69.0 to 97.1% saturation) Measured in all replicates on Day 0, and in alternating replicates from each level daily thereafter.
Test Temperature: 25 ± 2°C Should be measured weekly in each chamber.	Daily: 25 to 27°C Continuous: 24 to 28°C Measured in all replicates on Day 0, and in alternating replicates from each level daily thereafter. In addition, temperature was continuously monitored in the control replicate A.
Photoperiod: 14 hr light/10 hr dark with 15- to 30-minute transition periods	16-hour light, 8-hour dark photoperiod, with 15- to 30-minute transition periods. Intensity ranged from 21 to 29 foot candles (220 to 370 lux).
Dosing Apparatus: 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations 3) A dilution factor not greater than 0.5 and controls should be used.	1) Intermittent-flow proportional diluter 2) Five toxicant concentrations 3) A dilution factor of 0.5 and appropriate controls were included.

Guideline Criteria	Reported Information
Flow Rate: 1) Flow rates should provide ≥5 volume additions per 24 hr. 2) Flow splitting accuracy must be within 10%. 3) Meter systems calibrated before study and general operation checked twice daily during test period.	1) 7.9 volume additions/day 2) Flow splitting accuracy was reported to be 5%. 3) The function of the diluter system was monitored daily and a visual check was performed twice daily.
Test Vessels: Materials and equipment that minimize sorption. Test vessels should be loosely covered. **Retention Chambers:** Can be constructed with netting material of appropriate mesh size.	1) Glass aquaria, measuring 30 x 15 x 20 cm and equipped with a 10-cm high side drain that maintained a constant exposure solution volume of 4.5 L. It was not reported if test chambers were covered. Prior to pairing: glass Petri dishes, 10-cm diameter, 2-cm deep, to which a 14-cm high Nitex® screen collar (350-µm mesh size) was attached. The solution volume within the retention chambers was *ca.* 785 mL. Following pairing: 6-cm diameter Petri dishes, 1.5-cm deep, to which a 14-cm high Nitex® screen collar (350-µm mesh size) was attached. The solution volume within the pairing chambers was *ca.* 250 mL.
Aeration: Permitted if necessary to maintain DO.	None reported.

Comments: The exposure system was in proper operation for at least 24 hours prior to test initiation to allow for equilibration.

Chemical System:

Guideline Criteria	Reported Information
Concentrations: Concentration ranges should be selected to determine the concentration response curves, LC_{50} values, and MATC. Toxicant level should be measured at each level at 0, 7, 14, 21, and 28 days, and should not vary more than 20% among replicate test chambers.	Nominal: negative control, 0.090, 0.19, 0.38, 0.75 and 1.5 ng ai/L Mean-measured: <0.10 (control), 0.17, 0.21, 0.28, 0.44 and 1.4 ng ai/L Water samples were collected for analytical verification from alternate replicate vessels (all levels) on Days 0, 7, 14, 21, and 28. Excessive analytical variation (CV >20%) was observed at all treatment levels (25-96%). The reviewer calculated time-weighted average (TWA) concentrations were 0.17, 0.22, 0.28, 0.45, and 1.6 ng ai/L. Measured concentrations in the lowest treatment group were <0.10 (<LOQ) on Days 0 and 7 so the nominal value of 0.090 ng ai/L was used to calculate the mean-measured and TWA concentrations.
Solvents: 1) Should not exceed 0.1 mL/L in a flow-through system. 2) Following solvents are acceptable: triethylene glycol, methanol, acetone, ethanol.	N/A; a solvent was not used

Comments: Throughout the test high analytical variation was measured. However, concentrations were generally consistent for the overall exposure with the variability showing up between sampling intervals. For example, the expected concentration gradient was maintained for the most part (50% dilution series). The variability was likely to due a number of factors including, but not limited to, very low test concentrations (down to 100 parts per quadrillion) and the highly adsorptive nature of the test material.

Fourteen out of the 15 quality control samples yielded recoveries of 97.4 to 118% of nominal for the fortified 0.200, 0.400, and 1.50 ng ai/L levels. One QC sample was outside of the acceptable range (155% of nominal).

Test solutions were delivered to the diluter system using glass wool-packed saturator columns. Approximately 15% of the column's total volume was packed with glass wool and then coated with saturated bifenthrin (~1.0 g of bifenthrin diluted with 50.0 mL of acetone). The column was attached to a vacuum pump to draw the solution evenly through the column to uniformly coat the wool with bifenthrin and evaporate the acetone. Once all of the wool was evenly coated and the acetone was evaporated the column was detached from the vacuum pump and attached to a FLUID Metering, Inc. (FMI) Pump. The saturator columns delivered solutions of approximately 1.3 µg ai/L. A second FMI pump was attached to deliver a constant flow of column effluent at 0.21 mL/min into the diluter mixing chamber to dose the system.

A 28-day preliminary flow-through exposure was performed with 40 juvenile mysids per level (20 per replicate) at target bifenthrin concentrations of 0 (negative and solvent controls), 0.0012, 0.0036, 0.011, 0.033, and 0.10 ng ai/L. All except the highest nominal concentration were <LOQ, so no analytical verification was performed. The results did not show a dose response gradient but rather a flat response across concentrations, results were difficult to evaluate in the absence of measured concentrations. Therefore, a second preliminary exposure with concentrations within the method validation range was performed. Nominal concentrations were 0.090, 0.19, 0.38, 0.75, and 1.5 ng ai/L and the test was conducted under flow-through conditions. Overall survival ranged from 73 to 85% and post-pairing survival ranged from 84 to 96% and was comparable between the controls and treatment groups. Male length and weight and female length were significantly reduced at the highest treatment level relative to the pooled control (Dunnett's test, $p < 0.05$); female dry weight was not affected. Time to first brood, reproduction (offspring per female), and F1 survival were comparable between the controls and treatment groups.

In the laboratory report the selection of doses for the definitive study was based on these results.

"Significant effects were observed at the high dose (1.5 ng/L) for male and female length and male dry weight. Also, the effect gradient across concentrations closely resembled a dose response for all growth endpoints. Therefore, after consultation with the Study Sponsor, the nominal concentrations selected for the definitive exposure were 0.090, 0.19, 0.38, 0.75 and 1.5 ng/L." (See page 28).

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was performed according to U.S. EPA (FIFRA) Good Laboratory Practice Standards (40 CFR, Part 160) with the following exceptions: routine food and dilution water contaminant screening analyses were not conducted according to GLP Standards, but were performed using a certified laboratory and standard U.S. EPA analytical methods.
Controls: 1) Survival of the first-generation controls (between pairing and test termination) must not be less than 70%. 2) At least 75% of the paired 1st generation females in the controls produced young or 3) The average number of young produced by the 1st generation females in the control(s) was at least 3.	<u>All validity requirements were fulfilled:</u> 1) post-pairing survival was 94% in the negative control group. 2) 100% of the paired 1st generation negative control females produced young. 3) An average of 18.3 offspring per female were produced by the 1st generation negative control female.
Data Endpoints must include: 1) The number of dead adult mysids on Days 7, 14, 21, and 28. Concentration-response curves, LC_{50} values, and associated 95% C.I. for each interval. 2) Body length of male and female mysids at the time of sexual discernment and again on Day 28. 3) Time to fist brood release. 4) Cumulative young per female. 5) If available, mortality, number of each sex, and body lengths of each sex should be recorded for the offspring. 6) Any abnormal behavior or appearance	**Endpoints evaluated in this study included:** 1) Post-pairing (i.e., Days 13-28) survival of adult mysids (male, female and combined sexes) and overall (Days 0-28) survival. 2) Body lengths and dry weights of male and female mysids at Day 28. 3) Not evaluated, but data provided 4) Percent of females producing young and no. offspring/female 5) 96-hour survival of offspring 6) Any abnormal behavior or appearance
Raw data included?	Yes, sufficient

Effects Data:

Adult (F_0)

Toxicant Conc. (ng ai/L)		Percent Survival (Mean ± SD)			
Nominal	TWA	Post-Pairing (Days 13-28)			Overall (Days 0-28) Combined
		Male	Female	Combined	
Control	<LOQ[(a)]	89 ± 7	98 ± 5	94 ± 5	87 ± 6
0.090	0.17	94 ±13	100 ± 0	98 ± 5	85 ± 14
0.19	0.22	94 ± 14	95 ± 10	93 ± 9	84 ± 10
0.38	0.28	97 ± 6	92 ± 9	93 ± 5	83 ± 3
0.75	0.45	92 ± 10	98 ± 4	95 ± 6	88 ± 5
1.5	1.6	89 ± 16	93 ± 8	93 ± 5	86 ± 13

[(a)] LOQ = 0.10 ng ai/L.

No statistically-significant differences from the negative control were detected for sex-specific post-pairing survival, combined sex post-pairing survival, or overall survival (Fisher's Exact test with Bonferroni-Holm adjustment, $p > 0.05$). Post-pairing control survival averaged 89, 98, and 94% for males, females, and combined sexes, respectively. Overall survival averaged 87% in the negative control and ranged from 83 to 88% in the treatment groups.

No behavioral abnormalities were observed during the study.

Toxicant Conc. (ng ai/L)		Reproduction (mean ± SD)		
Nominal	TWA	Percent of Females Producing Young	Days to First Brood Release	Number of Offspring per Female
Control	<LOQ[(a)]	100 ± 0	17.8 ± 0.8	18.3 ± 3.4
0.090	0.17	95 ± 10	18.2 ± 1.0	15.0 ± 1.4
0.19	0.22	100 ± 0	19.8 ± 1.2	12.8 ± 5.4

Toxicant Conc. (ng ai/L)		Reproduction (mean ± SD)		
0.38	0.28	100 ± 0	17.7 ± 0.9	14.9 ± 4.2
0.75	0.45	100 ± 0	18.3 ± 0.7	14.4 ± 2.6
1.5	1.6	100 ± 0	18.8 ± 2.3	13.8 ± 3.4

[(a)] LOQ = 0.10 ng ai/L.

The time to first brood release was not evaluated as a toxicological endpoint. Reviewer-calculated times to first brood release ranged from 17.7 in the TWA 0.28 ng ai/L treatment group to 19.8 in the TWA 0.22 ng ai/L treatment group. Offspring were produced at all treatment levels and reproduction (offspring per female) averaged 18.3 in the negative control and ranged from 12.8 (0.22 ng ai/L) to 15.0 (0.17 ng ai/L) with no statistical differences detected between the control and treatment groups (Williams Multiple Comparison Test, $p > 0.05$). The NOAEC and LOAEC values based on reproduction were 1.6 and >1.6 ng ai/L, respectively.

Toxicant Conc. (ng ai/L)		Growth (mean ± SD)			
Nominal	TWA	Total Body Length (mm)		Dry Body Weight (mg)	
		Male	Female	Male	Female
Control	<LOQ[(a)]	7.37 ± 0.17	7.57 ± 0.16	0.85 ± 0.021	1.18 ± 0.054
0.090	0.17	7.10* ± 0.14	7.47 ± 0.16	0.80 ± 0.044	1.14 ± 0.12
0.19	0.22	7.08* ± 0.13	7.54 ± 0.076	0.79* ± 0.031	1.15 ± 0.087
0.38	0.28	7.22 ± 0.12	7.58 ± 0.071	0.81 ± 0.023	1.13 ± 0.13
0.75	0.45	7.21 ± 0.070	7.59 ± 0.03	0.83 ± 0.031	1.13 ± 0.11
1.5	1.6	7.15* ± 0.083	7.53 ± 0.13	0.83 ± 0.045	1.17 ± 0.015

[(a)] LOQ = 0.10 ng ai/L

* Statistically-significant difference compared to the negative control ($p < 0.05$) based on Dunnett's Multiple Comparison Test. However, the study author did not feel that these reductions were biologically relevant due to a lack of a clear toxicant-related concentration-response.

Terminal growth of F_0 mysids was not affected for males or females at any treatment level relative to the negative control. Significant reductions (Dunnett's Multiple Comparison Test, $p < 0.05$) in male body length were detected at the TWA 0.17, 0.22, and 1.6 ng ai/L treatment groups. The calculated minimum significant difference value was 2.9%, which was considered low for this endpoint. Further, there did not appear to be dose-dependent relationship, suggesting that these inhibitions were not treatment related. Male body weight was significantly reduced at the TWA 0.22 ng ai/L treatment level (Dunnett's Multiple Comparison Test, $p < 0.05$) but as with male length did not exhibit a dose-dependent response and was not considered treatment-related. The NOAEC and LOAEC values for sex-specific growth were 1.6 and >1.6 ng ai/L, respectively.

Offspring (F_1)

Toxicant Conc. (ng ai/L)		Percent Survival (Mean ± SD) 96 Hours
Nominal	Mean-measured	
Control	<LOQ[a]	93 ± 10
0.090	0.17	98 ± 5
0.19	0.22	100 ± 0
0.38	0.28	90 ± 12
0.75	0.45	100 ± 0
1.5	1.6	93 ± 5

[a] LOQ = 0.0061 to 0.0075 µg/L.
[b] Data from two replicates.

No treatment-related effect on F_1-generation survival was observed following a 96-hour observation period, with mean survival rates of 93 to 100% for all control and treatment levels, and no statistically-significant differences from the negative control indicated at any level. The NOAEC for offspring survival was 1.6 ng ai/L based upon mean-measured concentrations.

Statistical Results:

Statistical Method: Endpoints that were statistically-analyzed included 28-day survival, male and female post-pairing survival, growth of males and females (dry body weight and total length), reproduction (number of young released per female), and 96-hour offspring survival.

Data for survival endpoints were analyzed using Fisher's Exact Test with Bonferroni-Holm's Adjustment. Reproduction and growth data were checked for normality using the Shapiro-Wilk's Test and for homogeneity of variance using Bartlett's Test. All endpoints met both assumptions; therefore, Dunnett's Multiple Comparison Test or William's Multiple Comparison Test was used for control comparisons. All statistical conclusions were made at the 95% level of certainty except in the basic assumption tests (e.g., Shapiro-Wilk's Test and Bartlett's Test), in which the 99% level of certainty was applied.

The NOAEC and LOAEC were based on significance data. All analyses were performed using CETIS™ (2013) statistical software and mean-measured concentrations.

Endpoint	Method	NOAEC	LOAEC	MATC

F_0 Survival (28 days)	Fisher's Exact Test with Bonferroni-Holm Correction	1.4 ng ai/L	>1.4 ng ai/L	Not reported
F_0 Survival, Male	Fisher's Exact Test with Bonferroni-Holm Correction	1.4 ng ai/L	>1.4 ng ai/L	Not reported
F_0 Survival, Female	Fisher's Exact Test with Bonferroni-Holm Correction	1.4 ng ai/L	>1.4 ng ai/L	Not reported
Offspring/female	Dunnett's Multiple Comparison Test	1.4 ng ai/L	>1.4 ng ai/L	Not reported
Total Length, Male	Dunnett's Multiple Comparison Test	1.4 ng ai/L	>1.4 ng ai/L	Not reported
Total Length, Female	Dunnett's Multiple Comparison Test	1.4 ng ai/L	>1.4 ng ai/L	Not reported
Dry Weight, Male	Dunnett's Multiple Comparison Test	1.4 ng ai/L	>1.4 ng ai/L	Not reported
Dry Weight, Female	Dunnett's Multiple Comparison Test	1.4 ng ai/L	>1.4 ng ai/L	Not reported
F_1 Survival (96 hr)	Fisher's Exact Test with Bonferroni-Holm Correction	1.4 ng ai/L	>1.4 ng ai/L	Not reported

Comments: None

12. REVIEWER'S STATISTICAL RESULTS:

Statistical Method: The reviewer analyzed F_0 survival (pre-pairing, post-pairing, overall), reproduction, time to first brood, sex-specific growth (i.e., length, weight), and F_1 survival. Data were tested for normality using the Shapiro-Wilk W test (α=0.01) and for homogeneity of variance using Bartlett's test (α=0.01). All endpoints except F_1 survival met these assumptions and were analyzed using the Dunnett Multiple Comparison test; F_1 survival was analyzed using the Mann-Whitney U Two-Sample test. All analyses were conducted using CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/2014. All statistical tests were conducted using the reviewer-calculated time-weighted average (TWA) concentrations at $\alpha = 0.05$ unless specified otherwise. Minor differences in mean survival values between the reviewer's and study author's calculations were due to non-treatment related mortalities. The reviewer corrected the number of initial mysids when appropriate (pre-pairing vs post-pairing) to accurately reflect survival rates.

Most sensitive endpoint: none

Endpoint	Method	NOAEC (µg ai/L)	LOAEC (µg ai/L)
F_0 Survival (pre-pairing)	Dunnett	1.6	>1.6
F_0 Survival (post-pairing)	Dunnett	1.6	>1.6
F_0 Survival (28 days)	Dunnett	1.6	>1.6
Time to first brood	Dunnett	1.6	>1.6
Offspring/female	Dunnett	1.6	>1.6
Female Length	Dunnett	1.6	>1.6
Female Weight	Dunnett	1.6	>1.6
Male Length	Dunnett	1.6	>1.6
Male Dry Weight	Dunnett	1.6	>1.6
F_1 Survival	Mann-Whitney	1.6	>1.6

General Comments: The reviewer's results were comparable to those reported by the study author. The reviewer found significant inhibitions in male dry weight at the 0.22 ng ai/L treatment level (7.08% reduction, $p = 0.038$) and in male length at the 0.17, 0.22, and 1.6 ng ai/L treatment levels (3.05-4.03% reduction, $p \leq 0.0397$). However, inhibitions were generally low (<10%) relative to the negative control and responses did not appear to be dose-dependent. Therefore, the reviewer did not consider these reductions to be biologically significant.

The reviewer calculated time-weighted average concentrations using the following equation:

where:
C TWA is the time-weighted average concentration,
C j is the concentration measured at time interval j (j = 0, 1, 2,...n)
t j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j
(e.g., t 0 = 0 hours (test initiation), t 1 =24 hours, t 2 =96 hours)

The reviewer's results were based on the TWA concentrations whereas the study author's results were based on the mean-measured concentrations. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

13. REFERENCES:

Fournier, A. 2013. Bifenthrin- Acute Toxicity to Mysids (*Americamysis bahia*) Under Flow-Through Conditions, Following OCSPP Draft Guideline 850.1035. Smithers Viscient, Wareham, MA. Study No. 14011.6105.

Ives, Michael. 2013. CETIS, Comprehensive Environmental Toxicity Information System™. User's Guide. Tidepool Scientific Software, McKinleyville, CA.

Mount, D.I., and W.A. Brungs. 1967. A simplified dosing apparatus for fish toxicological studies. *Water Research.* 1:21-29.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. *Water Research.* 3:793-821.

Wei-hong, Y.E. 2004. Effects of bifenthrin on Daphnia magna during chronic toxicity test and recovery test. *Journal of Environmental Sciences* Vol. 16, No. 5, pp 843-846.

Batch ID:	05-0791-4564	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	06 Feb-14	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Seawater
Ending Date:		**Species:**	Americamysis bahia	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Lab In-House Culture	**Age:**	<24h
Sample ID:	06-1066-6442	**Code:**	49412102	**Client:**	CDM Smith
Sample Date:	06 Feb-14	**Material:**	Bifenthrin	**Project:**	Insecticide
Receive Date:		**Source:**	Consumer Specialty Products (CONTASKF		
Sample Age:	NA	**Station:**			

Batch Note: PC Code 128825 MRID 49412102

Sample Note: PC Code 128825 MRID 49412102

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-1943-1464	F0 Female Dry Weight	1.6	>1.6	NA	13.8%		Dunnett Multiple Comparison Test
15-3008-4599	F0 Female Length	1.6	>1.6	NA	2.56%		Dunnett Multiple Comparison Test
15-4035-1657	F0 Male Dry Weight	0.17	0.22	0.1934	6.68%		Dunnett Multiple Comparison Test
20-0202-1959	F0 Male Length	<0.17	0.17	NA	2.9%		Dunnett Multiple Comparison Test
12-8778-6778	F0 Survival Entire Study	1.6	>1.6	NA	18.4%		Dunnett Multiple Comparison Test
20-4619-5954	F0 Survival Post Pairing	1.6	>1.6	NA	17.8%		Dunnett Multiple Comparison Test
08-2081-1207	F0 Survival Pre Pairing	1.6	>1.6	NA	10.0%		Dunnett Multiple Comparison Test
11-7492-6539	F1 Survival	1.6	>1.6	NA	10.1%		Mann-Whitney U Two-Sample Test
13-9227-5812	n Offpspring Per Female	1.6	>1.6	NA	33.7%		Dunnett Multiple Comparison Test
03-3811-3549	Time to First Brood	1.6	>1.6	NA	12.2%		Dunnett Multiple Comparison Test

F0 Female Dry Weight Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	1.18	1.09	1.27	1.14	1.26	0.0278	0.0556	4.72%	0.0%
0.17		4	1.14	0.945	1.32	0.96	1.22	0.0597	0.119	10.5%	3.61%
0.22		4	1.15	1.01	1.29	1.03	1.22	0.0438	0.0876	7.61%	2.34%
0.28		4	1.13	0.924	1.33	1.01	1.31	0.0641	0.128	11.4%	4.25%
0.45		4	1.14	0.956	1.32	0.99	1.26	0.0569	0.114	10.0%	3.4%
1.6		4	1.17	1.15	1.19	1.15	1.18	0.00707	0.0141	1.21%	0.64%

F0 Female Length Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	7.58	7.32	7.83	7.37	7.71	0.0806	0.161	2.13%	0.0%
0.17		4	7.47	7.22	7.72	7.24	7.58	0.078	0.156	2.09%	1.42%
0.22		4	7.55	7.43	7.66	7.49	7.65	0.0366	0.0733	0.97%	0.43%
0.28		4	7.58	7.47	7.69	7.48	7.63	0.0344	0.0688	0.91%	-0.03%
0.45		4	7.59	7.54	7.64	7.56	7.63	0.0147	0.0294	0.39%	-0.17%
1.6		4	7.53	7.33	7.74	7.34	7.61	0.0643	0.129	1.71%	0.59%

F0 Male Dry Weight Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0.848	0.81	0.885	0.83	0.88	0.0118	0.0236	2.79%	0.0%
0.17		4	0.798	0.725	0.87	0.75	0.85	0.0229	0.0457	5.73%	5.9%
0.22		4	0.788	0.738	0.837	0.76	0.83	0.0155	0.031	3.93%	7.08%
0.28		4	0.815	0.777	0.853	0.78	0.83	0.0119	0.0238	2.92%	3.83%
0.45		4	0.828	0.786	0.869	0.79	0.85	0.0131	0.0263	3.18%	2.36%
1.6		4	0.835	0.768	0.902	0.79	0.89	0.021	0.042	5.03%	1.47%

F0 Male Length Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	7.38	7.1	7.65	7.2	7.59	0.0866	0.173	2.35%	0.0%
0.17		4	7.1	6.87	7.34	6.97	7.24	0.0738	0.148	2.08%	3.73%
0.22		4	7.08	6.87	7.29	6.91	7.19	0.0645	0.129	1.82%	4.03%
0.28		4	7.22	7.02	7.42	7.04	7.32	0.0616	0.123	1.71%	2.13%
0.45		4	7.22	7.1	7.33	7.13	7.3	0.0352	0.0705	0.98%	2.2%
1.6		4	7.15	7.02	7.28	7.04	7.23	0.0403	0.0806	1.13%	3.05%

F0 Survival Entire Study Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0.873	0.785	0.96	0.833	0.95	0.0275	0.055	6.3%	0.0%
0.17		4	0.85	0.625	1	0.643	0.947	0.0707	0.141	16.6%	2.67%
0.22		4	0.838	0.685	0.991	0.75	0.947	0.048	0.096	11.5%	4.03%
0.28		4	0.828	0.775	0.88	0.8	0.875	0.0165	0.0329	3.98%	5.17%
0.45		4	0.877	0.803	0.951	0.813	0.923	0.0232	0.0464	5.29%	-0.43%
1.6		4	0.857	0.645	1	0.714	1	0.0668	0.134	15.6%	1.78%

F0 Survival Post Pairing Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0.873	0.785	0.96	0.833	0.95	0.0275	0.055	6.3%	0.0%
0.17		4	0.894	0.623	1	0.643	1	0.0852	0.17	19.1%	-2.42%
0.22		4	0.838	0.685	0.991	0.75	0.947	0.048	0.096	11.5%	4.03%
0.28		4	0.828	0.775	0.88	0.8	0.875	0.0165	0.0329	3.98%	5.17%
0.45		4	0.935	0.861	1	0.889	1	0.0233	0.0467	4.99%	-7.13%
1.6		4	0.918	0.801	1	0.824	1	0.0366	0.0733	7.99%	-5.11%

F0 Survival Pre Pairing Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0.972	0.921	1	0.944	1	0.016	0.0321	3.3%	0.0%
0.17		4	0.956	0.861	1	0.875	1	0.0296	0.0592	6.19%	1.71%
0.22		4	1	1	1	1	1	0	0	0.0%	-2.86%
0.28		4	1	1	1	1	1	0	0	0.0%	-2.86%
0.45		4	0.939	0.828	1	0.875	1	0.0351	0.0701	7.46%	3.38%
1.6		4	0.933	0.771	1	0.786	1	0.0507	0.101	10.9%	4.08%

F1 Survival Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0.925	0.773	1	0.8	1	0.0479	0.0957	10.4%	0.0%
0.17		4	0.975	0.895	1	0.9	1	0.025	0.05	5.13%	-5.41%
0.22		4	1	1	1	1	1	0	0	0.0%	-8.11%
0.28		4	0.9	0.716	1	0.8	1	0.0577	0.115	12.8%	2.7%
0.45		4	1	1	1	1	1	0	0	0.0%	-8.11%
1.6		4	0.925	0.845	1	0.9	1	0.025	0.05	5.41%	0.0%

n Offpspring Per Female Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	18.3	12.9	23.7	14.8	21.6	1.7	3.4	18.6%	0.0%
0.17		4	15	12.7	17.2	13.6	16.6	0.699	1.4	9.36%	18.3%
0.22		4	12.8	4.19	21.4	5.4	18.4	2.7	5.41	42.3%	30.1%
0.28		4	14.9	8.19	21.6	9	18.4	2.11	4.22	28.3%	18.6%
0.45		4	14.4	10.2	18.5	11.6	17.4	1.32	2.64	18.4%	21.6%
1.6		4	13.8	8.41	19.1	10.2	18	1.68	3.36	24.4%	24.9%

Time to First Brood Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	18	16.7	19.3	17	19	0.408	0.816	4.54%	0.0%
0.17		4	18.3	16.2	20.3	17	20	0.629	1.26	6.89%	-1.39%
0.22		4	19.8	17.7	21.8	18	21	0.629	1.26	6.37%	-9.72%
0.28		4	17.8	16.2	19.3	17	19	0.479	0.957	5.39%	1.39%
0.45		4	18.5	17.6	19.4	18	19	0.289	0.577	3.12%	-2.78%
1.6		4	18.8	15.2	22.3	17	22	1.11	2.22	11.8%	-4.17%

F0 Female Dry Weight Detail

C-ng ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1.26	1.15	1.16	1.14
0.17		0.96	1.22	1.2	1.16
0.22		1.03	1.14	1.21	1.22
0.28		1.09	1.01	1.1	1.31
0.45		1.12	1.26	1.18	0.99
1.6		1.17	1.18	1.15	1.18

F0 Female Length Detail

C-ng ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	7.71	7.7	7.53	7.37
0.17		7.24	7.51	7.55	7.58
0.22		7.5	7.54	7.65	7.49
0.28		7.48	7.63	7.59	7.62
0.45		7.58	7.63	7.59	7.56
1.6		7.59	7.61	7.34	7.59

F0 Male Dry Weight Detail

C-ng ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	0.88	0.83	0.85	0.83
0.17		0.77	0.82	0.85	0.75
0.22		0.76	0.83	0.77	0.79
0.28		0.82	0.83	0.78	0.83
0.45		0.85	0.84	0.83	0.79
1.6		0.89	0.84	0.79	0.82

F0 Male Length Detail

C-ng ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	7.59	7.44	7.28	7.2
0.17		6.97	7.24	7.22	6.98
0.22		6.91	7.19	7.05	7.17
0.28		7.26	7.32	7.04	7.26
0.45		7.23	7.3	7.2	7.13
1.6		7.04	7.18	7.16	7.23

F0 Survival Entire Study Detail

C-ng ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	0.875	0.95	0.833	0.833
0.17		0.643	0.933	0.947	0.875
0.22		0.765	0.75	0.889	0.947
0.28		0.824	0.875	0.813	0.8
0.45		0.923	0.813	0.882	0.889
1.6		0.714	0.938	1	0.778

F0 Survival Post Pairing Detail

C-ng ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	0.875	0.95	0.833	0.833
0.17		0.643	0.933	1	1
0.22		0.765	0.75	0.889	0.947
0.28		0.824	0.875	0.813	0.8
0.45		0.923	0.929	1	0.889
1.6		0.909	0.938	1	0.824

F-0 Survival Pre-pairing

C-ng ai/L	Control Type	Rep	Rep 2	Rep 3	Rep 4
0	Negative Control	1	1	0.944	0.944
0.17		1	1	0.947	0.875
0.22		1	1	1	1
0.28		1	1	1	1
0.45		1	0.875	0.882	1
1.6		0.786	1	1	0.944

F1 Survival Detail

C-ng ai/L	Control Type	Rep	Rep 2	Rep 3	Rep 4
0	Negative Control	1	1	0.8	0.9
0.17		1	0.9	1	1
0.22		1	1	1	1
0.28		0.8	1	1	0.8
0.45		1	1	1	1
1.6		0.9	0.9	1	0.9

n Offpspring Per Female Detail

C-ng ai/L	Control Type	Rep	Rep 2	Rep 3	Rep 4
0	Negative Control	21.6	20.8	16	14.8
0.17		13.6	16.6	14	15.6
0.22		13.6	**5.4**	18.4	13.8
0.28		17.4	14.8	9	18.4
0.45		12.8	11.6	17.4	15.6
1.6		14.6	18	10.2	12.2

Time to First Brood Detail

C-ng ai/L	Control Type	Rep	Rep 2	Rep 3	Rep 4
0	Negative Control	17	18	18	19
0.17		20	18	17	18
0.22		21	20	18	20
0.28		17	18	19	17
0.45		18	19	19	18
1.6		18	17	22	18

Table 1. Preliminary Life-cycle exposure of mysids (*Americamysis bahia)* to bifenthrin – First generation (F_0) survival

Nominal Concentration (ng/L)	Replicate	Male Survival (%)	Female Survival (%)	Post-Pairing Survival (%)	28-Day Survival (%)
Control	A	100	88	93	76
	B	67	100	87	81
	Mean	83	94	90	79
Solvent Control	A	100	86	94	84
	B	75	100	87	87
	Mean	88	93	90	85
Pooled Control		85	93	90	82
0.090	A	89	100	93	87
	B	80	92	88	88
	Mean	84	96	91	87
0.19	A	56	100	73	69
	B	100	90	94	89
	Mean	78	95	84	79
0.38	A	89	100	93	87
	B	88	80	83	75
	Mean	88	90	88	81
0.75	A	75	86	80	67
	B	89	89	89	80
	Mean	82	87	84	73
1.5	A	100	80	92	79
	B	100	100	100	88
	Mean	100	90	96	83

Table 2. Preliminary Life-cycle exposure of mysids *(Americamysis bahia)* to bifenthrin – Mean total body length and dry weight of first generation (F_0) male and female mysids

Nominal Concentration (ng/L)	Replicate	Total Length {mm}		Dry Weight {mg}	
		Male	Female	Male	Female
Control	A	7.13	7.39	0.84	1.20
	B	7.08	7.34	0.83	1.13
	Mean	7.10	7.37	0.84	1.16
Solvent Control	A	7.18	7.69	0.80	1.23
	B	7.04	7.37	0.88	1.28
	Mean	7.11	7.53	0.84	1.26
Pooled Control		7.11	7.45	0.84	1.21
0.090	A	7.02	7.47	0.86	1.36
	B	6.92	7.29	0.82	1.13
	Mean	6.97	7.38	0.84	1.24
0.19	A	6.68	7.33	0.80	1.17
	B	6.82	7.08	0.80	1.17
	Mean	6.75	7.20	0.80	1.17
0.38	A	6.95	7.26	0.81	1.15
	B	6.82	7.04	0.82	1.00
	Mean	6.89	7.15	0.82	1.07
0.75	A	6.74	7.22	0.76	1.23
	B	6.76	7.20	0.83	1.18
	Mean	6.75	7.21	0.80	1.20
1.5	A	6.48	6.92	0.76	1.03
	B	6.68	7.22	0.75	1.15
	Mean	6.58"	7.07"	0.76"	1.09

Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test.

Table 3. Preliminary Life-cycle exposure of mysids *(Americamysis bahia)* to bifenthrin – First generation (F_0) reproductive success (offspring per female)

Nominal Concentration (ng/L)	Replicate	Time to Maturation (days)	Time to First Brood Release (days)	Offspring Per Active Females	Percentage of Reproductively
Control	A	10	16.0	23.4	100
	B	10	15.8	25.2	100
	Mean	IO	15.9	24.3	IOO
Solvent Control	A	11	16.0	25.4	100
	B	10	16.8	22.6	100
	Mean	11	16.4	24.0	100
Pooled Control		IO	16.2	24.2	100
0.090	A	II	18.0	14.6	100
	B	10	17.0	19.2	100
	Mean	11	17.5	16.9	100
0.19	A	10	I7.4	12.6	100
	B	10	16.6	24.2	100
	Mean	IO	17.0	18.4	100
0.38	A	10	19.2	14.2	100
	B	10	17.0	14.4	100
	Mean	IO	18.1	14.3[a]	IOO
0.75	A	11	18.8	16.6	100
	B	11	17.6	25.0	100
	Mean	11	18.2	20.8	100
1.5	A	11	19.2	14.8	100
	B	10	17.8	18.8	80
	Mean	11	18.5	16.8	90

[a] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test.